



PATENT

Our Docket P41 9408

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF APPEALS AND INTERFERENCES

In re Application of:)
Wahl and O'Gorman) Group Art Unit: 1804
Serial No.: 08/147,912) Examiner: C. Low
Filed: November 3, 1995)
For: FLP-MEDIATED GENE)
MODIFICATION IN)
MAMMALIAN CELLS, AND)
COMPOSITIONS AND CELLS)
USEFUL THEREFOR)

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9/29/95
Date of Signature

APPELLANTS' BRIEF ON APPEAL

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PATENT
Our Docket: P41 9498

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF APPEALS AND INTERFERENCES

In re application of:)
Wahl and O'Gorman) Group Art Unit: 1814
)
) Examiner: C. Low
Serial No.: 08/147,912)
)
Filed: November 3, 1993)
)
For: FLP-MEDIATED GENE MODIFICATION)
IN MAMMALIAN CELLS, AND)
COMPOSITIONS AND CELLS USEFUL)
THEREFOR)

Assistant Commissioner for Patents
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Washington, D.C. 20231

APPELLANTS' BRIEF UNDER 37 CFR 1.192

Sir:

I. INTRODUCTION

This is an appeal from a decision of the Examiner dated July 14, 1994, finally rejecting pending claims 25, 26, 28, 42-46 and 48 in the above-identified patent application. Notice of Appeal was timely filed January 17, 1995. The Appeal Brief is being submitted in triplicate (an original and two copies) as required by 37 C.F.R. § 1.192(a).

II. REAL PARTY IN INTEREST

The real party in interest in this appeal is The Salk Institute for Biological Studies, 10010 North Torrey Pines Road, La Jolla, California 92037.

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III. RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences that will directly affect, or be directly affected by, or have a bearing on the Board's decision in this appeal.

IV. STATUS OF THE CLAIMS ON APPEAL

The present application, USSN 08/147,912, filed November 3, 1993, is a file-wrapper continuation of USSN 07/666,252, filed March 8, 1991.

The original application ('252) contained claims 1-59. Claims 1-24, 29-41 and 56-59 were withdrawn from consideration as a result of Appellants' election in response to the Requirement for Restriction mailed July 17, 1992.

By an amendment and request for reconsideration filed March 12, 1993 in connection with '252, claims 25-28, 42-44, 47-51 and 54-55 were amended. On June 3, 1993, the Examiner issued an Office Action finally rejecting claims 25-28 and 42-55.

On October 18, 1993, Appellants filed an amendment after final and request for reconsideration wherein claims 25, 26, 28, 42-46 and 48 were amended and claims 27, 47 and 49-55 were cancelled.

An Advisory Action was issued October 26, 1993 in connection with '252, stating that Appellants' request for reconsideration was considered but was deemed not to overcome the

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grounds for rejection. The amendments proposed on October 18, 1993 were not entered.

On November 3, 1993, Appellants filed the present application as a file-wrapper-continuation of original application '252. In the concurrently filed preliminary amendment, non-elected claims 1-24, 29-41 and 56-59 were cancelled, and the amendments proposed on October 18, 1993 in connection with '252 were entered in the present application. Thus claims 25, 26, 28, 42-46 and 48 are pending in the present application.

By an amendment and request for reconsideration filed May 9, 1994, claims 25, 26, 28, 42-44 and 48 were further amended. On July 14, 1994, the Examiner issued an Office Action finally rejecting claims 25, 26, 28, 42-46 and 48.

Appellants filed a Notice of Appeal on January 20, 1995, from the final rejection of claims 25, 26, 28, 42-46 and 48. On July 20, 1995, Appellants filed an Amendment After Final and request for reconsideration wherein proposed amendments to claims 25, 26, 42, 44 and 48 were presented.

An Advisory Action was issued on August 24, 1995, stating that Appellants' request for reconsideration was considered but was deemed not to overcome the grounds for rejection of the claims and that upon the filing of an Appeal, the proposed amendments to claims 25, 26, 42, 44 and 48, proposed in the July 20, 1995 Amendment After Final would not be entered.

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Accordingly, pending claims 25, 26, 28, 42-46 and 48, as they stood prior to Appellants' July 20, 1995 Amendment After Final, define the subject matter of this Appeal. A copy of claims 25, 26, 28, 42-46 and 48 is presented in Appendix A.

V. STATUS OF AMENDMENTS

An Amendment After Final under 37 C.F.R. § 1.116 was submitted on July 20, 1995. Amendments to claims 25, 26, 42, 44 and 48 were proposed in response to the issues raised in the Official Action dated July 14, 1994. In the Advisory Action issued on August 24, 1995, Appellants' request for reconsideration was deemed not to overcome the grounds for rejection of the claims and that upon the filing of an Appeal, the amendments to claims 25, 26, 42, 44 and 48, proposed on July 20, 1995, would not be entered.

VI. SUMMARY OF INVENTION

In accordance with the present invention, there are provided methods for the site-specific integration of DNA into the genome of a cell. The specific site of integration is referred to as a "FLP recombination target site" (i.e., an "FRT"). Because the FRT is a relatively short sequence of nucleotides, it can be integrated into the DNA of a host without unduly disrupting the host's normal processes. Once the FRT is integrated at a confirmed site of interest, employing standard techniques, the targeted integration of a desired construct provided by the FLP/FRT recombination system used in accordance with the claimed methods alleviates the randomness commonly associated with the transfection of DNA.

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The novel site-specific recombination system of the present invention provides the artisan with the ability to target the integration of transfected DNA to specific chromosomal sites in mammalian host cells at frequencies substantially exceeding those of both random and other site-specific integration systems. Additionally, invention recombination system allows for immediate confirmation and analysis of the recombination event. The recombination system described herein is distinctive in its precision and predictability, providing methods which enable the artisan to routinely create or disrupt functional translational reading frames at intended sites of integration.

VII. ISSUES

1. Are claims 25, 26, 28, 42-46 and 48 supported by a specification which provides a reasonable written description, enablement and best mode for practicing the claimed invention, as required by 35 U.S.C. § 112, first paragraph?
2. Are claims 25, 26, 28, 42-46 and 48 enabled for precisely targeting DNA to a predetermined site of integration?
3. Are claims 25, 26, 28, 42-46 and 48 sufficiently definite so as to satisfy the requirements of 35 U.S.C. § 112, second paragraph?
4. Are claims 25 and 28 anticipated by Golic et al., Cell 69:499-509 (1989) under 35 U.S.C. § 102(b)?
5. Are claims 25 and 28 obvious over Golic et al. under 35 U.S.C. § 103?

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6. Are claims 25, 26, 28, 42-46 and 48 obvious over Sauer, U.S. Patent No. 4,959,317 (1990) taken with Golic et al. under 35 U.S.C. § 103?

VIII. GROUPING OF CLAIMS

Consistent with the Examiner's treatment of claims 25 and 28 separately from the other pending claims, claims 25, 26 and 28 should be considered separately from claims 42-46 and 48.

IX. ARGUMENT

1. **The specification provides adequate written description, enablement and best mode for practicing the claimed invention.**

The Examiner's assertion that the specification fails to provide a reasonable written description, enablement and best mode for practicing the claimed invention is respectfully submitted to be in error for the following reasons. The invention process is clearly described as a two step process, wherein FLP recombination sites are introduced in step one, and the targeted introduction of a desired construct is then accomplished in step two. Introduction in step two is targeted to the FLP recombination site introduced in step one, and is mediated by FLP recombinase.

Integration of the initial FLP recombination target site is not targeted. Appellants' invention requires only that the initial FLP recombination site integrate within a chromosome. Those of skill in the art can readily identify numerous means by

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which short fragments of DNA, such as FLP recombination target sites, can be chromosomally integrated. The precise site of integration can then be readily determined using standard detection assays, i.e., Southern Blot Analysis, histochemical screening, and the like. Once the initial FLP recombination target site is chromosomally integrated, this serves as the target site for subsequent site-specific integration of nucleic acid sequences of interest.

It is respectfully submitted that Appellants have provided substantial enablement for the practice of the present invention. For example, Appellants teach the introduction of an initial FLP recombination target site into a cell by way of a first transfection step. See Example 1, pages 17 through 22 of Appellants' specification. Appellants further teach that the initial FLP recombination target site, once chromosomally integrated, serves as a target site for the precise integration of subsequent nucleic acids having at least one FLP recombination target site.

It is hornbook law that Appellants can act as their own lexicographers. Appellants have described the claimed process as "precisely" targeted integration, referring specifically to the precise nature of step two of the claimed process. Appellants' claims must be read in light of the specification. It is inappropriate for the Examiner to ignore the clear teachings of Appellants' disclosure by invoking his own reading of the language employed.

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2. The disclosure fully enables precisely targeting DNA to a predetermined site.

The Examiner's assertion that the disclosure is not enabled for precisely targeting DNA to a predetermined site is respectfully submitted to be in error for the following reasons. As noted above, the claimed method of integration comprises two steps, i.e., introduction of FLP recombination target site(s), followed by targeted integration of DNA mediated by FLP recombinase. It is respectfully submitted that Appellants have detailed the methods of the invention in the specification such that a person of ordinary skill in the art could readily reproduce and practice the claimed invention. Accordingly, it is respectfully submitted that the claims under examination are, indeed, enabled in the specification such as to allow one of ordinary skill in the art to practice the claimed invention without undue experimentation.

3. Claims 25, 26, 28, 42-46 and 48 fully satisfy the requirements of 35 U.S.C. § 112, second paragraph.

The Examiner's assertion that claims 25, 26, 28, 42-46 and 48 are indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as invention is respectfully submitted to be in error for the following reasons. The Examiner's concern with precise targeting of the first DNA is respectfully submitted to be misplaced. As discussed above, the invention process occurs in two steps. First, a FLP recombination target site is introduced, then a nucleic acid is precisely targeted and integrated at the previously introduced FLP recombination target site.

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The proposed amendments to claim 42, as submitted herewith, as part of the Amendment After Final, render moot the concern that reference to "nucleic acid" in the preamble of the claim refers to both the first and second nucleic acids. It is respectfully submitted to be clear that the precise targeting contemplated by the present claims is accomplished in the FLP recombinase-promoted step.

4. Claims 20 and 28 are not anticipated by Golic et al.

The Examiner's assertion that claims 25 and 28 are anticipated by Golic et al. is respectfully submitted to be in error for the following reasons.

Appellants' invention, as defined by claims 25 and 28, requires precisely targeted integration of a nucleic acid into the genome of a mammalian host cell. In contrast, Golic et al. relates to excision of nucleic acid from the genome in insect cells. Since anticipation requires the presence, in the cited reference, of all elements of an invention as set forth in the claims, Golic et al. clearly does not anticipate the present invention, as defined by claims 25 and 28.

5. Claims 25 and 28 are not rendered obvious by Golic et al.

The Examiner's assertion that Golic renders claims 25 and 28 obvious is respectfully submitted to be in error for the following reasons. Contrary to the Examiner's assertion, Golic et al. do not disclose or suggest the use of the FLP

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recombination system in mammalian cells. The cited passage of Golic referring to mammalian systems (at page 499, column 2), relates to the use of a completely different recombination system, i.e., the bacteria-derived system, Cre lox.

Appellants respectfully disagree with the Examiner's assertion that it would have been obvious from the Golic disclosure to expect the Golic process to function in other organisms. It is respectfully submitted that the cited reference must be considered for all it discloses, and for what it would suggest to those of skill in the art. The acknowledgement by the authors that homologous recombination is rare and difficult to control must be considered. Moreover, one must also consider the substantial complexity of the human genome relative to the stark simplicity of the *Drosophila* genome (employed by Golic).

The increased complexity of the mammalian genome is reflected in the presence of at least a 30-fold increase in the amount of nucleotide sequence in the mammalian genome, relative to the amount of nucleotide sequence in *Drosophila* genome. In addition, the mammalian genome is further complicated by the presence of substantial quantities of repetitive sequence. The dramatically increased size of the mammalian genome, relative to *Drosophila*, makes it statistically more likely that target sequences competitive with FLP recombinase target sites would exist. The presence of such competing target sites would severely limit the ability of the invention method to work. Indeed, it was surprising, in view of the simple systems disclosed in the prior art, to discover that the recombination system employed in the practice of the present invention functions very efficiently in mammalian cells.

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The "introducing" step(s) contemplated by Golic comprises a mating step whereby a construct (comprising a marker gene placed between FRT repeats) is provided by one gamete and an expression vector for the production of FLP recombinase is provided by the other gamete. There is no requirement for two different DNAs to interact, as required by Appellants' claims. FLP recombinase is merely called upon to promote an intramolecular process whereby marker gene is excised from between two predisposed FLP sites. It is far different, and far more complex, to introduce a construct into a single predisposed FLP site, as required by Appellants' claims. Golic does not disclose or suggest such introduction of DNA.

Appellants respectfully disagree with the Examiner's assertion that the Golic authors expected the recombination system described therein to work in other organisms. It is respectfully submitted that the statement by Golic et al. that "we expect that it will work in other organisms as well" is mere unsupported conjecture that *Drosophila* is not the only system in which the tested recombination system would be expected to function. There is clearly no disclosure or suggestion, however, that reference to "other organisms" embraces organisms as complex as mammalian systems. Indeed, there is a substantial difference between *Drosophila* and mammalian cells. It is only with benefit of Appellants' disclosure, which teaches the first successful use of FLP recombinase-based recombination system in mammalian cells, that the reference can be read to embrace mammalian cells. Such use of Appellants' disclosure is clearly improper.

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6. **Claims 25, 26, 28, 42-26 and 48 are not rendered obvious by Sauer taken with Golic.**

The Examiner's assertion that claims 25, 26, 28, 42-46 and 48 are unpatentable over Sauer taken with Golic is respectfully submitted to be in error for the following reasons. Sauer does not disclose or suggest the use of FLP recombination systems. Instead, Sauer describes a very different recombination system, i.e., the bacteria-derived Cre lox system. It is respectfully submitted that a bacteria-derived system provides no guidance as to the performance of a completely different, yeast-derived recombination system. The discovery that the Cre lox system functions in mammalian cells provides no guidance as to whether a completely different recombination system, i.e., the FLP-based system, will function in mammals.

The invention recombination system further distinguishes over Sauer by requiring integration of nucleic acid into the genome of a host cell. In contrast, the Sauer Cre lox system operates in mammals as part of an extrachromosomal element. Accordingly, Sauer is unable to provide guidance to one who wishes to employ a very different recombination system in mammals. Indeed, absent Appellants' disclosure, there is no suggestion in the art that any recombination system, other than Cre lox, could be used in mammalian cells.

Further reliance on Golic is unable to cure the deficiencies of Sauer. Indeed, the asserted combination of references is respectfully submitted to be improper as both references deal with different recombination systems, derived from different sources (i.e., bacteria or yeast), and which

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operate by different mechanisms (i.e., as extrachromosomal elements or integrated into the genome). Accordingly, the combination of Sauer taken with Golic is unable to render obvious the present claims.

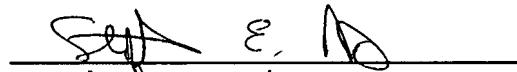
X. APPENDIX

Appendix A contains a copy of pending claims 25, 26, 28, 42-46 and 48 that are the subject of the present appeal.

XI. CONCLUSION

In view of the above remarks, it is respectfully submitted that claims 25, 26, 28, 42-46 and 48 are in condition for allowance. Accordingly, it is respectfully requested that the decision of the Examiner, finally rejecting claims 25, 26, 28, 42-26 and 48 be reversed.

Respectfully submitted,



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APPENDIX A

The text of pending claims on appeal are:

25. (Amended) A method for precisely targeting integration of a nucleic acid into the genome of a mammalian host cell, said method comprising:

- (i) stably integrating a first nucleic acid comprising a FLP recombination target site (FRT) into the genome of said mammalian host cell,
- (ii) introducing into said mammalian host cell of step (i) a second nucleic acid comprising at least one FRT along with an FLP recombinase, wherein said FLP recombinase catalyzes recombination between the integrated FRT and the FRT of said second nucleic acid, thereby precisely targeting integration of said second nucleic acid into the genome of said mammalian host cell of step (i).

26. (Amended) A method for excising a second nucleic acid that has been integrated into the genome of a mammalian host cell according to the method of Claim 25, comprising contacting the genomic DNA of said mammalian host cell with an FLP recombinase, wherein said FLP recombinase catalyzes recombination of the FRT of said first nucleic acid and the FRT of said second nucleic acid, thereby excising the integrated second nucleic acid from the genome of said mammalian host cell.

28. (Amended) A method according to Claim 25, further comprising introducing into the mammalian host cell of step (ii) a third nucleic acid comprising at least one FRT, along with an FLP recombinase, wherein said FLP recombinase catalyzes recombination between an integrated FRT with the FRT of said third nucleic acid, thereby precisely targeting integration of said third nucleic acid into the genome of said mammalian host cell.

42. (Amended) A method for the site-specific integration of a nucleic acid into the genome of a mammalian cell wherein at least one FRT is stably integrated in the genome of said mammalian cell, said method comprising:

introducing into said mammalian cell a first nucleic acid comprising at least one FRT and at least a first partial coding sequence of a first gene of interest, along with an FLP recombinase, wherein the FLP recombinase catalyzes recombination between the integrated FRT and the FRT of said first nucleic acid, thereby specifically integrating said first nucleic acid at the site of FRT recombination in said genome of the mammalian cell.

43. (Amended) A method according to Claim 42, wherein said FRT(s) integrated in the genome of said mammalian cell is/are positioned within the protein coding sequence of said gene of interest.

44. (Amended) A method according to Claim 42, further comprising contacting said mammalian cell with a second nucleic acid comprising at least one FRT and at least a second partial coding sequence of the first gene of interest or a partial coding sequence of a second gene of interest, along with an FLP recombinase, wherein the FLP recombinase catalyzes recombination between said integrated FRT and the FRT of said second nucleic acid, wherein said second nucleic acid specifically integrates at the site of FRT recombination in reading frame with said first nucleic acid, wherein the combination of said first and said second nucleic acids provides a functional gene.

45. A method according to Claim 42 wherein said FLP recombinase is provided by a FLP expression vector.

46. A method according to Claim 45 wherein the expression of FLP recombinase by said FLP expression vector is subject to regulatory control.

48. (Amended) A method according to Claim 42, further comprising contacting said mammalian cell with a second nucleic acid comprising at least one FRT, along with an FLP recombinase, wherein the FLP recombinase catalyzes recombination between said integrated FRT and the FRT of said second nucleic acid, wherein said second nucleic acid specifically integrates at the site of FRT recombination and combines with said first nucleic acid, wherein the combination of said first nucleic acid and said second nucleic acid prevents expression of the first gene of interest.